

## SEQUENCE LISTING

<110> Schmidt, James J.  
Stafford, Robert G.

<120> High Throughput Assays for the Proteolytic Activities  
of Clostridial Neurotoxins

<130> 003/224/SAP

<140> 09/962,360

<141> 2000-09-25

<150> US 60/235,050

<151> 2001-09-25

<160> 12

<170> Apple Macintosh Microsoft Word 6.0

<210> 1

<211> 17

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide chosen such that it is cleaved by  
BoNT A

<220>

<221> misc\_feature

<222> 8 and 14

<223> Xaa at 8 is N(epsilon)-2,4-(dinitrophenyl)-lysine and  
Xaa at 14 is S-(fluoresceinyl)-cysteine

<400> 1

Ser	Asn	Arg	Thr	Arg	Ile	Asp	Xaa	Ala	Asn	Gln	Arg	Ala	Xaa	Arg
1				5					10					15

Met Leu

<210> 2

<211> 17

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide chosen such that it is cleaved by

BoNT A

<220>

<221> misc\_feature

<222> 11 and 14

<223> Xaa at 11 is N(epsilon)-2,4-(dinitrophenyl)-lysine and

Xaa at 14 is S-(7-dimethylamino-4-methyl-coumarin-3-

carboxamidomethyl)-cysteine

<400> 2

Ser	Asn	Arg	Thr	Arg	Ile	Asp	Glu	Ala	Asn	Xaa	Arg	Ala	Xaa	Arg
1				5					10					15

Met Leu

<210> 3

<211> 35

<212> PRT

<213> Artificial sequence

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<223> synthetic peptide chosen such that it is cleaved by

BoNT B

<220>

<221> misc\_feature

<222> 14 and 20

<223> Xaa at 14 is N(epsilon)-2,4-(dinitrophenyl)-lysine and  
Xaa at 20 is S-(fluoresceinyl)-cysteine

<400> 3

Leu	Ser	Glu	Leu	Asp	Asp	Arg	Ala	Asp	Ala	Leu	Gln	Ala	Xaa	Ala
1				5					10					15
Ser	Gln	Phe	Glu	Xaa	Ser	Ala	Ala	Lys	Leu	Lys	Arg	Lys	Tyr	Trp
				20					25					30
Trp	Lys	Asn	Leu	Lys										
				35										

<210> 4

<211> 35

<212> PRT

<213> Artificial sequence

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<223> synthetic peptide chosen such that it is cleaved by  
BoNT B

<220>

<221> misc\_feature

<222> 17 and 20

<223> Xaa at 17 is N(epsilon)-2,4-(dinitrophenyl)-lysine and  
Xaa at 20 is S-(7-dimethylamino-4-methyl-coumarin-3-  
carboxamidomethyl)-cysteine

<400> 4

Leu	Ser	Glu	Leu	Asp	Asp	Arg	Ala	Asp	Ala	Leu	Gln	Ala	Gly	Ala
1				5					10					15
Ser	Xaa	Phe	Glu	Xaa	Ser	Ala	Ala	Lys	leu	Lys	Arg	Lys	Tyr	Trp
				20					25					30

Trp Lys Asn Leu Lys  
35

<210> 5

<211> 39

<212> PRT

<213> Artificial sequence

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<223> synthetic peptide chosen such that it is cleaved by  
BoNT D or BoNT F

<220>

<221> misc\_feature

<222> 19 and 25

<223> Xaa at 19 is N(epsilon)-2,4-(dinitrophenyl)-lysine and  
Xaa at 25 is S-(fluoresceinyl)-cysteine

<400> 5

Ala	Gln	Val	Asp	Glu	Val	Val	Asp	Ile	Met	Arg	Val	Asn	Val	Asp
1				5					10					15
Lys	Val	Leu	Xaa	Arg	Asp	Gln	Lys	Leu	Xaa	Glu	Leu	Asp	Asp	Arg
				20					25					30
Ala	Asp	Ala	Leu	Gln	Ala	Gly	Ala	Ser						
				35										

<210> 6

<211> 39

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide chosen such that it is cleaved by  
BoNT D or BoNT F

<220>

<221> misc\_feature

<222> 22 and 25

<223> Xaa at 22 is N(epsilon)-2,4-(dinitrophenyl)-lysine and  
Xaa at 25 is S-(7-dimethylamino-4-methyl-coumarin-3-  
carboxamidomethyl)-cysteine

<400> 6

Ala Gln Val Asp Glu Val Val Asp Ile Met Arg Val Asn Val Asp  
1 5 10 15  
Lys Val Leu Glu Arg Asp Xaa Lys Leu Xaa Glu Leu Asp Asp Arg  
20 25 30  
Ala Asp Ala Leu Gln Ala Gly Ala Ser  
35

 $\langle 210 \rangle$  7

<211> 39

<212> PRT

<213> Artificial sequence

 $\langle 220 \rangle$ 

<223> synthetic peptide chosen such that it is cleaved by BoNT D or BoNT F

 $\langle 220 \rangle$ 

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<221> misc_feature
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<222> 22 and 25

<223> Xaa at 22 is N(epsilon)-2,4-(dinitrophenyl)-lysine and  
Xaa at 25 is 2-amino-3-(7-methoxycoumarin-4-yl)-propionic  
acid

<400> 7

Ala Gln Val Asp Glu Val Val Asp Ile Met Arg Val Asn Val Asp  
1 5 10 15  
Lys Val Leu Glu Arg Asp Xaa Lys Leu Xaa Glu Leu Asp Asp Arg  
20 25 30  
Ala Asp Ala Leu Gln Ala Gly Ala Ser  
35

<210> 8

<211> 24

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide chosen such that it is cleaved by

BoNT A

<220>

<221> misc\_feature

<222> 1

<223> Xaa at 1 is N-fluoresceinyl-glycine

<400> 8

Xaa	Gly	Gly	Ser	Asn	Arg	Thr	Arg	Ile	Asp	Glu	Ala	Asn	Gln	Arg
1				5					10					15
Ala	Thr	Arg	Met	Leu	Gly	Gly	Gly	Cys						
				20										

<210> 9

<211> 42

<212> PRT

<213> Artificial sequence

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<223> synthetic peptide chosen such that it is cleaved by

BoNT B

<220>

<221> misc\_feature

<222> 1

<223> Xaa at 1 is N-fluoresceinyl-glycine

<400> 9

Xaa	Gly	Gly	Leu	Ser	Glu	Leu	Asp	Asp	Arg	Ala	Asp	Ala	Leu	Gln
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

1	5	10	15
Ala Gly Ala Ser Gln Phe Glu Thr Ser	Ala Ala Lys Leu Lys Arg		
	20	25	30
Lys Tyr Trp Trp Lys Asn Leu Lys Gly	Gly Gly Cys		
	35	40	

<210> 10

<211> 46

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide chosen such that it is cleaved by  
BoNT D or BoNT F

<220>

<221> misc\_feature

<222> 1

<223> Xaa at 1 is N-fluoresceinyl-glycine

<400> 10

Xaa Gly Gly Ala Gln Val Asp Glu Val Val Asp Ile Met Arg Val	
1	5 10 15
Asn Val Asp Lys Val Leu Glu Arg Asp Gln Lys leu Ser Glu Leu	
	20 25 30
Asp Asp Arg Ala Asp Ala Leu Gln Ala Gly Ala Ser Gly Gly Gly	
	35 40 45

Cys

<210> 11

<211> 116

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide chosen such that it is cleaved by  
BoNT E

<220>

<221> misc\_feature

<222> 1

<223> Xaa at 1 is S-fluoresceinyl-cysteine

<400> 11

Xaa	Asn	Lys	Leu	Lys	Ser	Ser	Asp	Ala	Tyr	Lys	Lys	Ala	Trp	Gly
1				5					10					15
Asn	Asn	Gln	Asp	Gly	Val	Val	Ala	Ser	Gln	Pro	Ala	Arg	Val	Val
			20						25					30
Asp	Glu	Arg	Glu	Gln	Met	Ala	Ile	Ser	Gly	Gly	Phe	Ile	Arg	Arg
			35						40					45
Val	Thr	Asn	Asp	Ala	Arg	Glu	Asn	Glu	Met	Asp	Glu	Asn	Leu	Glu
			50						55					60
Gln	Val	Ser	Gly	Ile	Ile	Gly	Asn	Leu	Arg	His	Met	Ala	Leu	Asp
			65						70					75
Met	Gly	Asn	Glu	Ile	Asp	Thr	Gln	Asn	Arg	Gln	Ile	Asp	Arg	Ile
			80						85					90
Met	Glu	Lys	Ala	Asp	Ser	Asn	Lys	Thr	Arg	Ile	Asp	Glu	Ala	Asn
			95						100					105
Gln	Arg	Ala	Thr	Lys	Met	Leu	Gly	Ser	Gly	Cys				
			110						115					

<210> 12

<211> 116

<212> PRT

<213> Artificial sequence

<220>

<223> synthetic peptide chosen such that it is cleaved by

BoNT E

<220>

<221> misc\_feature

<222> 1

<223> Xaa at 1 is S-fluoresceinyl-cysteine

<400> 12

Xaa	Asn	Lys	Leu	Lys	Ser	Ser	Asp	Ala	Tyr	Lys	Lys	Ala	Trp	Gly
1				5					10					15
Asn	Asn	Gln	Asp	Gly	Val	Val	Ala	Ser	Gln	Pro	Ala	Arg	Val	Val
			20						25					30
Asp	Glu	Arg	Glu	Gln	Met	Ala	Ile	Ser	Gly	Gly	Phe	Ile	Arg	Arg



				35					40					45
Val	Thr	Asn	Asp	Ala	Arg	Glu	Asn	Glu	Met	Asp	Glu	Asn	Leu	Glu
				50					55					60
Gln	Val	Ser	Gly	Ile	Ile	Gly	Asn	Leu	Arg	His	Met	Ala	Leu	Asp
				65					70					75
Met	Gly	Asn	Glu	Ile	Asp	Thr	Gln	Asn	Arg	Gln	Ile	Asp	Arg	Ile
				80					85					90
Met	Glu	Lys	Ala	Asp	Ser	Asn	Lys	Thr	Arg	Ile	Asp	Glu	Ala	Asn
				95					100					105
Gln	Ala	Ala	Thr	Lys	Met	Leu	Gly	Ser	Gly	Cys				
				110					115					